CHEMICAL CHANGES ACCOMPANYING ABSCISSION IN COLEUS BLUMEI

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 240

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Introduction

VON MOHL (13) in 1860 was the first to announce that previous to the fall of the leaf there is formed near the base of the petiole a definite separation layer in which abscission always occurs by the separation of the cells from each other with their walls still intact. The xylem tubes not being included in this separation layer are finally ruptured mechanically, and the leaf falls. He also called attention to the fact that abscission and the formation of a protective tissue are two very distinct processes, and that the latter process might either precede or follow the former. WIESNER (17) in 1871 confirmed the observation of VON MOHL in the main, and formulated the theory that the dissolution of the intercellular substance of the cells of the separation layer is caused by the action of organic acids developed in the leaves. In 1886 MOLISCH (14) suggested that a gum ferment might be the cause of this dissolution process. Two years later MANGIN (11), upon his discovery of the pectic nature of the middle lamella of cell walls in plants, indirectly advanced the knowledge of abscission. Since this discovery the abscission process has generally been referred to as a dissolution of the pectose and calcium pectate of the middle lamella. LLOYD (8, 9), assuming the organic acid theory of WIESNER, speaks of the process as a hydrolysis of these pectic compounds, and later (11) of cellulose also.

The anatomical workers disagree somewhat on the amount of the cell wall altered during abscission. LEE (7) in 1911 reported the disappearance of the middle lamella only, and two years later HANNIG (6) reported the same condition in the abscission of flowers, with the exception of a species of *Mirabilis* and of *Oxybaphus*, in which the entire cell wall disappeared. On the other hand, TISON

(15) as early as 1900, working with numerous species studied later by LEE, stated that in general the secondary membranes of the cell wall also undergo alteration and disappear, leaving only the thin tertiary membrane lining the cell lumen. LLOYD (9, 10) has recently found the same condition in the abscission of cotton bolls and also in *Mirabilis*, instead of the disappearance of the entire cell wall as reported by HANNIG. It seems improbable that the presence of weak organic acids would be sufficient to account for this extreme alteration of the cellulose walls of these cells. Certain authors have suggested that the catalytic effect of enzymes may be an important factor in abscission, but experimental evidence has been wanting.

Aside from his organic acid theory, WIESNER (21) in 1905 suggested increased turgor as a cause of abscission under conditions of forced leaf-fall. FITTING (5) in 1911 accepts this view to account for the rapid abscission of petals when forced. The suggestion lacks experimental confirmation, and the work of HANNIG (6) indicates less need for its assumption.

The external factors capable of accelerating leaf-fall are extremely diversified. These have been summarized in the main by LLOYD (8). The more important are high and low light intensity, high and low water supply, high temperatures and frost, low concentrations of anesthetics, toxic concentrations of acids and salts, and wounding of the blade. On the other hand, low concentrations of oxygen and high concentrations of anesthetics retard leaf-fall, a state of rigor being produced by the latter.

The internal changes affected by these various external factors have received very little critical study. The work discussed in the present paper was undertaken to determine some of the internal changes accompanying abscission of leaves in *Coleus Blumei* var. Golden Bedder. This plant was chosen for study partly on account of its ease of propagation, but mainly for its simplicity of analysis owing to the absence of protective tissue at the time of abscission.

Anatomy

In order to appreciate fully the chemical changes taking place in the abscission layer, it is necessary not only to compare the

abscission layer with the adjacent regions of the petiole, but also to follow the changes in the abscission layer itself from the time of its formation to the fall of the leaf. This latter process is most easily accomplished in *Coleus* by beginning with the terminal bud and taking the leaves as they appear in order down the stem, from the youngest to the oldest. On this basis the following description is applicable to *Coleus* plants growing in 4-inch pots under greenhouse conditions, with each plant bearing 8 pairs of leaves, and with the eighth pair in the process of abscissing.

In the first 2 pairs of leaves below the terminal bud there is no evidence of an abscission layer. The cells in the region where the abscission layer is later to occur are in the enlargement period of growth. The formation of the abscission layer in *Coleus* is usually initiated in the third pair of leaves below the terminal bud. Cell divisions in 2–4 layers of cells across the base of the petiole of these leaves begin in the epidermal and cortical region and gradually extend inward, reaching the phloem about the time the leaves appear as the fifth pair in order below the terminal bud. In the sixth pair of leaves the formation of the abscission layer is practically completed and involves all the tissue of the petiole except the xylem tubes. Growth of the leaf in general ceases long before this period is reached. In the majority of cases the fourth pair of leaves below the terminal bud are fully expanded. The formation of the abscission layer in *Coleus* therefore begins a short time before the maturity of the leaf and continues for a considerable period afterward. As a rule the layer is 8–12 cells in thickness. These cells always remain smaller than the neighboring cells of the adjacent regions of the petiole and their walls are somewhat thinner. At the time of abscission alteration of the walls of the cells of the abscission layer is quite general, but a continuous plane of separation is finally formed somewhat nearer the distal side of this layer. This extreme alteration of cell walls is localized in the abscission layer and is not found throughout the entire leaf, as was reported by WIESNER (18).

While this description is true in general for *Coleus* plants bearing 8 pairs of leaves, slight variations are not infrequent. The stages of development may be either retarded or accelerated. Under

conditions of forced leaf-fall all processes are greatly accelerated. The data in table I show that all processes, including the process of abscission, may be completed in the third pair of leaves in a period of 2 or 3 days as a result of the amputation of the blades. On the other hand, amputation of the blades of the first and second pairs of leaves before the beginning of the formation of the abscission layer inhibits its formation entirely.

Method of abscission

UNDER ORDINARY GROWING CONDITIONS.—The method of abscission has received much attention, but a critical survey of the exact changes in the cellulose and pectic compounds is wanting. An attempt to follow these changes in *Coleus* led to the discovery of certain facts which have a direct bearing upon the existing theories of the cause of abscission.

Microchemical analyses show that there is a breaking down not only of the calcium pectate of the middle lamella of the cells of the separation layer, but also of the cellulose of the secondary membrane, leaving only a thin layer of cellulose surrounding the lumen of the cells. This cellulose is first changed to pectose, which, according to CROSS (1), TOLLENS (16), and EULER (3), contains more oxygen than cellulose and probably is an oxidized form. The pectose is then further changed to pectin and pectic acid; the excess pectic acid becomes gelatinous and is no longer able to hold the cells together, and the leaf falls. The changes taking place in the walls of the xylem tubes are still to be investigated. During this process there is not a disappearance of calcium from the cell walls, but the excess of pectic acid produced renders the amount of calcium present insufficient to maintain the solidity of this portion of the cell wall. The excess pectic acid appears to come from the transformation of the pectose rather than from the breaking down of the calcium pectate of the middle lamella. Since this description differs from all previous accounts of the method of abscission, further discussion is postponed until all the facts are brought together under the topic of microchemical analysis.

FORCED LEAF-FALL.—Leaf-fall was accelerated by treatment with ethylene, amputation of the blade, and by allowing the soil

to become dry and then suddenly applying an excess of water. Under the first two treatments the leaves began to fall within 24 hours.

The rate of petiole fall after the amputation of the blade is shown in table I. At the beginning of the experiment each plant had 8 pairs of leaves and 1 blade of each pair was removed. The numbers refer to the total number of abscissed petioles at the corresponding dates.

TABLE I

RATE OF PETIOLE FALL FOLLOWING AMPUTATION OF BLADES

Plant	January					February		March							
	27	28	29	30	31	1	2	1	2	3	4	5	6	7	10
A*	1	5	....	....	....	....	6	6	....	....	....	....	....	....	6
B*	1	4	....	5	....	....	6	6	....	....	....	....	....	....	6
C†	....	....	....	....	....	....	....	....	3	4	6	....	....	6	....
D†	....	....	....	....	....	....	....	....	4	5	....	....	....	6	....
E†	....	....	....	....	....	....	....	....	4	6	6	....	....	6	....

* Blades amputated January 26; † blades amputated March 1.

A microchemical analysis of the abscission layer in all these cases showed exactly the same changes in cellulose and pectic substances as noted under ordinary conditions of growth. Furthermore, changes in oxidases, calcium in solution, and iron, to be discussed later, were the same in all cases. These facts emphasize again the need of experimental investigation before accepting the turgor pressure theory of the cause of abscission.

It is interesting to note that while the petioles usually absciss soon after amputation of the blade, there is one striking exception. If the blade is removed before the abscission layer is initiated, growth ceases throughout the entire petiole, the layer fails to develop, and the petiole is not dropped. The data in table I show that the 6 lowest petioles soon fall, but the upper 2 remain attached. This is an easy method of locating the period of formation of the abscission layer. This extreme cessation of growth in the petiole induced by artificial means has some features in common with a retardation of growth and abscission formation in the petioles of the upper leaves under natural conditions of flowering and fruiting. As a result of slowing up of growth and formation of abscission

layers in the petioles of the upper 3–5 pairs of leaves accompanying the development of the floral axis, these leaves remain on during the entire flowering and fruiting period. An investigation of the internal changes accompanying these 2 phenomena may throw some light upon abscission in general.

Organic acids as a cause of leaf-fall

WIESNER (**17–21**) cites three lines of experimental evidence as proof that the dissolution of the middle lamella is a result of the accumulation of organic acids in the aging leaves: (1) yellow leaves macerated and extracted with water when titrated were more acid than green leaves; (2) cuttings placed in 2.5 per cent oxalic acid dropped their leaves in a few days; (3) the exposed abscission surface of the petiole always gives an acid reaction to neutral red.

Abscission in *Coleus* when examined from the point of view of this theory shows several facts in disagreement and not one in its favor. Cuttings placed in non-toxic concentrations of oxalic acid showed no acceleration of leaf-fall over that of cuttings in distilled water. Cuttings in 0.0002 N oxalic acid showed slight toxic effects. The immersed part of the stem and the tips of young leaves on axillary branches became brown in color. The concentration of acid used by WIESNER was 1500 times as great, but he fails to state what plants were used and whether toxic effects were produced. Cuttings of *Coleus* in 0.0016 N oxalic acid did not show an acceleration of leaf-fall, although the toxic effects were strongly pronounced. It was further found that the plants soon became adjusted to the oxalic acid. Plants started in 0.0002 N oxalic acid were transferred every third day to a concentration of acid double that of the previous concentration. This was continued until the plants were finally placed in 0.0512 N oxalic acid. There was no acceleration of leaf-fall during the entire period. At the end of the treatment the cells of the plant were found to be filled with starch.

Similarly, potted plants infiltrated with non-toxic concentrations of oxalic acid showed no acceleration in leaf-fall. The plants were inverted under bell jars in vessels containing the various solutions, and the air was exhausted to 3 cm. of mercury. The volume of solution entering the infiltrated plant was approximately

equal to one-fourth the volume of the plant. No toxic effects were noted for concentrations of 0.0064 N oxalic acid and below. The results are given in table II.

TABLE II

SHOWING EFFECT OF DIFFERENT CONCENTRATIONS OF OXALIC ACID ON RATE OF ABSCISSION

Concentration	February														March				
	15	16	17	18	19	20	21	22	23	24	25	26	27	28	1	2	3	4	5
0.0256 N oxalic acid	..	..	..	..	..	..	..	..	..	2	..	3	..	4	5	..	6	..	..
0.0128 " " "	..	..	..	..	..	..	..	2	..	..	..	3	..	5	..	..	6	..	..
0.0064 " " "	1	..	..	..	..	..	..	2	..	3	..	..	..	4	5	..	6	..	..
0.0032 " " "	..	..	1	..	..	..	..	..	..	..	..	2	..	5	..	..	..	..	6
0.0016 " " "	..	..	..	..	..	..	..	..	..	1	..	..	..	2	3	4	5	6	..
0.0008 " " "	..	..	..	..	..	..	..	1	..	2	..	..	..	3	..	4	5	6	..
0.0004 " " "	..	..	..	..	..	..	..	..	..	2	..	..	..	4	6	7	..	8	..
Untreated	..	..	..	..	..	..	..	..	..	3	..	4	..	6	7	..	..	..	8
"	..	..	1	..	..	..	..	..	..	3	..	4	..	6	..	8	..	..	..

The plants were infiltrated February 9, and again February 22. Leaf-fall was allowed to occur normally under greenhouse conditions. The numbers refer to the total number of leaves off at the corresponding date.

Although concentrations of 0.0128 and 0.0256 N oxalic acid showed marked toxic effects, abscission was not accelerated; concentrations between 0.04 and 0.12 N oxalic acid killed many blades without killing the petioles and stem. In such cases abscission of the petioles occurred within 2 or 3 days, just as in the case of petiole fall after amputation of the blade, or severe wounding of the blade.

Likewise direct measurements of acidity do not agree with those of WIESNER. Table IV gives the acidity for 9 different regions of the plant. Falling leaves are not so acid as green leaves. Fresh yellow leaves in the act of abscissing when titrated with NaOH, using phenolphthalein as an indicator, had an acidity equivalent to 0.0069 cc. of normal acid per gram of wet weight. Fresh green leaves collected at the same time from the same plants had an acidity equivalent to 0.0089 cc. of normal acid per gram of wet weight. In both cases the leaves were weighed as rapidly as pos-

sible after collecting, macerated in a mortar, made up to volume with distilled water, and after shaking for 30 minutes the solutions were filtered through a Buchner funnel and definite portions taken for titration. Fresh abscission layers treated in this way had an acidity of 0.0100 cc. per gram of wet weight, while that of the adjacent part of the petiole was 0.0095 cc. These two figures are not to be compared with those preceding, as the two sets of titrations were made at different times and on different plants.

If the calcium pectate were being hydrolyzed by an organic acid, one would expect to find either an increase of calcium in solution in the cells of the abscission layer or an increase of crystals of calcium compounds in these cells. Such is not the case. Neither are there any calcium oxalate crystals in the middle lamella of these cells, such as one finds when the middle lamella is broken down by adding oxalic acid to sections under the microscope.

Finally, the marked acidity of the abscission surface of a falling leaf was certainly not correctly interpreted by WIESNER. He ascribes the acidity of the abscission surface to the excretion of organic acids from the interior of the cells. This abscission surface is a continuous layer of pectic acid, formed during the abscission process, and the acidity of the abscission surface, therefore, is a result of the formation of pectic acid during abscission, and not of the escape of acids previously formed in the cells. This acidity of the middle lamella to neutral red may be seen in *Coleus* in any part of the plant, and it is increased in the walls of the abscission layer only after the formation of pectic acid during abscission.

In conclusion, therefore, neither the turgor pressure theory nor the organic acid theory proposed by WIESNER to account for the cause of leaf-fall is in accordance with the facts observed in *Coleus*.

Effect of salts on leaf-fall

According to CZAPEK (2), the membranes of plant cells are colloidal in nature, and MANGIN has shown that the middle lamella is composed of pectic acid in combination with calcium. During the process of abscission the middle lamella undergoes a chemical alteration and the pectic acid present takes up water and swells. It was expected, therefore, that salts might show either a lyotropic

effect on the intake of water by the pectic acid or a specific effect of salt formation with this acid and thus affect the course of abscission. The following anions were used in the form of their potassium and calcium salts: PO_4, SO_4, Cl, NO_3, and CNS; and the following cations in the form of their chlorides: K, Na, Ca, Ba. In all cases 0.01 normal concentrations were used. The plants were treated by infiltration, by placing cuttings directly in the solutions, and by adding the salts to the soil. Abscission was slightly accelerated by treating the plants with 4 parts of ethylene per million of air. In all cases the results were the same. Neither lyotropic nor specific effects were noted. Similarly, concentrations of potassium and calcium chlorides between 0.04 and 0.00016 normal showed no marked effect.

The experiment was repeated under conditions of rapid acceleration of abscission by treating the plants with 700 parts of ethylene per million of air. The results which agree with those above are summarized in table III.

TABLE III

SHOWING EFFECT OF SALTS ON ABSCISSION

CONCENTRATION	FEBRUARY 12	FEBRUARY 15		FEBRUARY 14		FEBRUARY 13	
	5 P.M.	9 A.M.	5 P.M.	9 A.M.	5 P.M.	9 A.M.	5 P.M.
0.01 N KH_2PO_4		1		3	5		5
" " K_2SO_4			2	3	4		4
" " $CaSO_4$			5	6			6
" " KCl			3	4	5		5
" " KNO_3		2	3	5			5
" " $Ca(NO_3)_2$				2		4	4
" " KCNS		1		3		4	4
Untreated		2			3	4	4
"	2	5	6				6
"				2		3	3
"		1		4		6	6
"		3		4		6	6
0.01 N KCl			3	4	5		5
" " NaCl			3	4	4		4
" " $CaCl_2$				4	4		4
" " $BaCl_2$			2	4	4		4

Cuttings in solutions of the different salts gave similar results. The great diversity of the checks noted in this table is very unusual.

The failure of calcium to show a specific effect was unexpected. Later work, however, has thrown some light upon the matter, and it will be discussed under microchemical analysis.

Oxygen pressure and leaf-fall

MOLISCH (14) grew plants half submersed in water and found that the aerial portions dropped their leaves sooner than the submersed portions. From this fact he concluded that low oxygen pressure retarded leaf-fall. This surmise proved to be correct. *Coleus* plants grown in a hydrogen atmosphere under bell jars with only sufficient oxygen to maintain a slow growth retain their leaves much longer than plants in normal air. Under conditions of the experiment the plants in normal air usually retain 8 pairs of leaves. In the hydrogen atmosphere the plant retained 11 pairs of leaves. Inception of decay at the base of the stem destroyed the experiment at this point. Similarly, petiole fall, after amputation of the blade, is greatly retarded in very low concentrations of oxygen. Plants grown in 0.1 normal oxygen pressure showed no retardation of leaf-fall. Whether the effect of oxygen in such cases is that of an essential factor influencing the general metabolism of the plant, or of a formative factor influencing directly the oxidase activity in the abscission layer, or of both acting simultaneously, is a problem still to be investigated. Likewise a critical investigation of the possibility of a double effect of carbon dioxide on leaf-fall might throw more light upon the causes underlying abscission.

Macrochemical analysis

In order to follow the chemical changes leading up to abscission, both macrochemical and microchemical methods of analysis were employed. About 2500 plants grown under greenhouse conditions were used for the macrochemical analysis. These plants were all grown at the same time under the same conditions, and collection of material was made at the same time each day. Series C was collected between February 17 and March 3, collections being taken from day to day as the lower leaves began to absciss. Series D and E were collected from these same plants on March 3 and 4.

Material when collected was placed in 70–80 per cent alcohol and heated to 70°C. for one hour to destroy enzymatic activity.

The material was then extracted with alcohol and ether. The residue was dried and analyzed for polysaccharides, calcium, and oxalates. The alcohol-ether extract was evaporated to dryness on a steam bath and then extracted with water at 70°C. The filtrate of this aqueous extract was analyzed for reducing and non-reducing sugars; ammonia, amino acid, and nitrate nitrogen; calcium in solution, and acidity.

Table IV gives a summary of an analysis of 9 different regions of the plant. Series C represents leaves in the act of abscissing, series E represents leaves at the time of the formation of the abscission layer, and series D represents leaves intermediate between these two points. Collection E1 represents approximately 5 mm. of the abscission end of the petiole, collection E2 an equal portion of the adjacent part of the petiole, and collection E3 a portion of the blades. In like manner, collections C1, C2, and C3 and collections D1, D2, and D3 represent these same three regions in their respective series.

Attention should be called to the fact that while collections C1, D1, and E1 represent the abscission end of the petiole, they do not represent the abscission layer only. In no case does the abscission layer represent more than about 5 or 6 per cent of the portion of the petiole taken. In collection C1 it represents still less, probably not more than 2 per cent, as in the abscissing leaf the petiole retains only about one-third of the abscission layer, the remaining two-thirds being attached to the stem.

It is evident that chemical changes in the abscission layer might be overshadowed by the remaining 95 per cent of the collection, and even more so in collection C1 than in collections D1 and E1. This is especially true of the nitrates, which are frequently confined almost entirely to the abscission layer and are more abundant in this layer at the time of abscission than at any other time, although the figures in the table might lead one to think they were most abundant a short time before abscission. As a matter of fact, a large percentage of the nitrates in the abscission layer of collection C1 were left in the part of the abscission layer remaining attached

TABLE IV

Collection	Percentage dry weight	Percentage of dry material			Expressed as percentage of total dry weight											Total acidity as normal acid (in cc.) per grain of wet weight
		Water soluble	Alcohol-ether soluble	Alcohol-ether but not water soluble	Total carbohydrates	Polysaccharides	Non-reducing disaccharides	Reducing substances	NH_3 nitrogen	Amino acid nitrogen	NO_3 nitrogen	Total calcium	Calcium in solution	Calcium not in solution	Oxalates as anhydrous oxalic acid	
E1	3.94	28.92	31.55	2.63	11.89	11.44	0.0	0.45	0.063	0.106	0.900	3.06	0.17	2.89	1.97	0.0068
E2	3.95	28.76	32.04	3.28	13.23	12.33	0.0	0.90	0.048	0.033	0.042	3.20	0.20	3.00	2.07	0.0079
E3	7.43	13.23	22.76	9.53	17.04	15.09	0.13	1.82	0.020	0.056	0.140	2.18	0.06	2.12	1.34	0.0057
D1	3.61	30.85	35.39	4.54	14.02	11.88	0.40	1.74	0.076	0.097	2.820	3.07	0.37	2.70	1.41	0.0073
D2	3.29	.....	33.32	.....	14.03	12.03	0.0	2.00	0.072	0.057	0.180	3.42	0.33	3.09	1.31	0.0058
D3	6.74	17.54	26.00	8.46	24.00	19.70	0.0	4.27	0.009	0.058	0.037	2.52	0.09	2.43	1.47	0.0100
C1	4.33	25.56	45.96	20.40	13.21	10.95	0.0	2.26	0.030	0.080	1.670	2.32	0.12	2.20	2.07	0.0069
C2	3.33	29.44	32.97	3.53	15.02	10.42	0.0	4.60	0.036	0.078	0.950	3.06	0.21	2.85	1.70	0.0055
C3	5.06	19.85	27.34	7.49	15.82	10.88	0.58	4.35	0.024	0.072	0.036	3.61	0.12	3.49	2.02	0.0065

to the stem, and therefore are not included in the analyses. The data in the table, therefore, represent only the general chemical changes during the life of the leaves, while the detailed chemical changes occurring in the abscission layer itself will be given under microchemical analysis. Should one desire to make a macrochemical analysis of the abscission layers alone in *Coleus* no less than 40,000 plants would be needed.

The data in table IV show an increase in dry weight in the abscission end of the petiole at the time of abscission; also an increase in alcohol-ether soluble material, but no increase in water soluble material. The significance of these changes is uncertain. In the older petioles there is a slight decrease in polysaccharides and an increase in reducing substances. There is no increase in ammonia and amino acids, as might be expected if the protoplasm were breaking down. Oxalates and total calcium remain fairly constant, but there is a slight decrease in the amount of calcium in solution and in the acidity. A more detailed discussion of the chemical changes in the abscission layer is given under microchemical analysis.

In the older blades there is a decided decrease in the amount of accumulated starch at the time of abscission, but the amount of reducing substances remains fairly constant. Attention has already been called to the fact that the formation of the abscission layer is completed while the leaf is still in an active photosynthetic condition. Both photosynthesis and the translocation of foods continue for several days or weeks later. The data clearly show that the presence of the abscission layer does not prevent the movement of water and foods between leaf and stem.

Microchemical analysis

A microchemical investigation of *Coleus* showed a striking localization of physical and chemical changes in the abscission layer shortly before and at the time of abscission. The formation of the abscission layer usually in the third pair of leaves and the occurrence of abscission usually in the eighth pair of leaves (when the plants are grown in 4-inch pots in a greenhouse) make it possible to study the whole history of the abscission layer by investigation of only 6

pairs of leaves in each plant. The fact that the leaves are opposite is also of advantage. Abscission of the pair may occur simultaneously, or one of the pair may absciss long before the other begins, or it may occur at any stage in between. Since both abscission layers at each node may readily be obtained in a single free-hand section, it is possible to contrast all stages of abscission under exactly the same treatment. A study of the changes induced by forcing abscission in one of the leaves at each node is likewise facilitated.

The investigations completed include a study of the distribution and amount of nitrates, carbohydrates, oxidases, iron, manganese, calcium in solution, and oxalates.

NITRATES.—The data in table IV show a great increase of nitrates in the abscission end of the petiole as compared with the remainder of the leaf. Furthermore, the nitrates in this part of the petiole are least abundant at the time of formation of the abscission layer and most abundant a short time before leaf-fall. As already noted, these figures cannot be taken to represent the percentage of nitrates in the abscission layer. Microchemical tests show some interesting variations. In many plants the increase in nitrates is confined almost entirely to the abscission layer, while in others the petiole or the neighboring part of the stem may also show a like increase. In all cases studied there is an increase in nitrates in the abscission layer just before and at the time of abscission. In some plants this increase is gradual from the time of the formation of the abscission layer to the time of abscission. In other cases only traces of nitrates appear in the abscission layer until a short time before abscission, when they increase rather suddenly.

CARBOHYDRATES.—The data in table IV show that the free reducing sugars, like the nitrates, increase in the abscission end of the petiole with the increase in the age of the leaves, but, unlike the nitrates, they are less abundant in this part of the petiole than in the remainder of the leaf. This correlation of the amount of reducing sugars and the age of the tissue is still more striking when studied microchemically. From the terminal bud to the oldest leaves there is a gradual increase in reducing sugars in both stems and leaves. This increase is initiated last in the abscission layer.

As a result the abscission layer has a lower percentage of reducing sugars throughout its entire history than the adjacent regions of the petiole. This difference is most marked in the fifth and sixth pairs of leaves near the close of the formation of the abscission layer, but it is still quite evident at the time of abscission. In the cell walls of the abscission layer the change in form of the carbohydrates is still more pronounced and significant. During the process of abscission the first evident change in the cell walls is a conversion of cellulose of the secondary cell membranes to pectose. The second step is a conversion of some of this pectose to pectic acid and pectin. This is followed by the breaking down of the middle lamella of calcium pectate and the separation of the cells. The changes from cellulose to pectose can readily be followed by differential staining and crystallization methods, and by solubility tests. The evidence of the conversion of pectose to pectin and pectic acid is based upon solubility tests. Pectin is soluble in water, pectic acid is insoluble in water but soluble in dilute alkalies, while pectose is insoluble in both water and dilute alkalies. When an abscission layer at the time of abscission is treated with 3 per cent ammonium or potassium hydroxide or with 5 per cent sodium carbonate, the free pectic acid is dissolved. If the walls are then again examined a considerable portion of the secondary membrane, bordering the middle lamella which is still intact, is seen to have disappeared. A discussion of the changes in the calcium pectate is postponed until all the remaining facts have been stated.

OXIDASES.—In the stem and petioles oxidases are found in the epidermal and phloem tissues. In the abscission layer oxidases are found in all tissues except the xylem. Not only is this distribution peculiar to this region, but the increase in oxidases with the age of the abscission layer is also quite pronounced. Quantitative tests of the increase in oxidative activity are still to be made.

IRON.—Slight traces of iron (Fe^{+++}) are usually found throughout the plant, especially where chlorophyll is present. It is most abundant in the xylem tubes and in the epidermal region until a few hours before abscission, when it becomes extraordinarily abundant in the cells of the abscission layer. The path of diffusion of

the iron leading to its accumulation in the abscission layer has not been traced. No manganese was found.

CALCIUM AND OXALATES.—The data in table IV show no marked difference in the distribution of oxalates in the leaves. Only occasional crystals of calcium oxalate are found in the cells, and none are found in the cell walls of the abscission layer at any time. Likewise the total calcium has a fairly constant distribution throughout the plant, but slight variations of the calcium in solution are to be noted. The most striking and significant changes of the amount of calcium in solution, however, are shown by microchemical tests. Treatment of sections with 50 per cent sulphuric acid or with 3 per cent oxalic acid or ammonium oxalate show an abundance of calcium in solution in all living cells of the petiole except those of the abscission layer at the time of abscission. The crystals of calcium sulphate or of calcium oxalate obtained by these treatments were very numerous in the cells of the abscission layer before the time of abscission, the latter averaging 30 crystals per cell, while during abscission only an occasional crystal was obtained. This decrease of calcium in solution is not always confined to the abscission layer, but breaks off rather abruptly in the first few layers of cells of the adjacent region of the petiole. In some cases cells not more than 5 cell layers distant from the line of cleavage showed no decrease in the number of crystals. These facts show that the calcium in solution in the abscission layer disappears during abscission, and it should be further stated that the disappearance takes place in the first stages of the process.

Summary of microchemical analysis

1. A pronounced increase in nitrates always occurs in the abscission layer at the time of abscission. This increase may be gradual, extending over the entire life-history of the abscission layer, or it may appear somewhat suddenly a short time before abscission.

2. A gradual increase in the amount of reducing sugars accompanies the aging of leaves and stem. This increase is initiated last and is least pronounced in the abscission layer.

3. During the process of abscission the cellulose of the secondary membrane of the cell walls of the abscission layer is converted into

pectose. This pectose is further transformed into pectic acid and pectin. The final stage is the breaking down of the calcium pectate of the middle lamella.

4. Oxidases are present in the epidermal and phloem tissues in both stems and petioles. In the abscission layer they are present in all tissues outside of the xylem, and increase in amount with the age of the abscission layer.

5. Slight traces of iron may be found in practically all parts of stem and petioles, but shortly before abscission there is a sudden accumulation of iron in the abscission layer.

6. The amount of oxalates remains fairly constant throughout the entire life of the leaves. There is no evidence of an increase of calcium oxalate crystals in the cells of the abscission layer at the time of abscission, nor are there any crystals of calcium oxalate in the walls of these cells.

7. Calcium in solution is abundant in all living cells of the plant except those of the abscission layer at the time of abscission, where it practically disappears.

Discussion

According to TOLLENS (**16**), pectose is an oxidized cellulose of the composition $9(C_6H_{10}O_5)-C_6H_{10}O_6$. The first step in the breaking down of the cell walls in abscission in *Coleus* is evidently one of oxidation of cellulose. This process is possibly a result of the accumulation and subsequent activity of oxidases in the abscission layer, and also of the catalytic action of iron on these oxidases. This may be merely an acceleration of the conversion of cellulose into pectose which ordinarily goes on in cell walls of plants with increasing age. Cellulases may play a part in this process, but the question is still to be investigated. EULER (**4**) succeeded in isolating a cellulase in a fungus, *Merulius lacrimans*, which was capable of altering cellulose, but cellulases in higher plants are still unknown. CZAPEK (**2**) and EULER (**3**) have called attention to the fact that our knowledge of cellulases is very limited.

The pectose formed from the cellulose is in turn readily transformed to pectic acid and pectin, and in this process the catalytic action of iron may again play an important rôle. Whether acids

and pectic enzymes also play a rôle in these changes is uncertain. Hydrolytic action may underlie some or all of these changes, but this must remain an open question until the molecular composition of these compounds is definitely known. At any rate, the transformation of the cellulose and pectose leads to the formation of an excess of pectic acid in the cell walls of the abscission layer.

The most important question still open is the cause of the final breaking down of the calcium pectate of the middle lamella. There appear to be but two possibilities. Either the calcium ion of the pectate is captured by some anion, liberated in the cells of the abscission layer, and held in solution or precipitated, thus freeing the pectic acid, or the breaking down of the cellulose and pectose may lead to such an excess of pectic acid that the available calcium is no longer able to hold a sufficient proportion of the pectic acid as a salt, and thus maintain the solidity of the middle portion of the cell wall.

The fact that calcium is not found in solution in the cells of the abscission layer, nor in crystalline forms either in the cells or in the cell walls, is decidedly against the first view, which is simply WIESNER'S organic acid theory stated in slightly different terms and which has already been discussed in detail.

The second view is more easily understood when we recall the well known law of physical-chemical equilibrium. As soon as an excess of pectic acid is present in contact with the calcium pectate of the middle lamella there is undoubtedly a diffusion of calcium ions from the middle lamella and a diffusion of pectic acid into the middle lamella until an equilibrium of distribution of the two ions is established. A critical proportion of pectic acid to calcium would be reached in the middle lamella when the excess pectic acid breaks the continuity of the calcium pectate layer. This second view has the further advantage of being in accordance with all the experimental facts so far known, particularly the formation of excess pectic acid in the cell walls and the paucity of calcium in solution in the cells of the abscission layer.

The fact already stated, in the discussion of calcium, that there is an abundance of calcium in solution in cells within 5 cell layers of the line of cleavage in abscission, indicates either that the process

of abscission is a very rapid one or that the diffusion of calcium from cell to cell is a very slow one. This fact also explains why the addition of calcium salts, already discussed, showed no specific effects on the rate of leaf-fall as the rate of diffusion of the calcium ions through the cells would again appear as a limiting factor.

Extensive comparative investigations of abscission in the light of facts discovered in *Coleus* are still to be made. TISON's statement that in general the secondary membranes also are altered in abscission indicates that these processes may be rather general, particularly since the reports of HANNIG and LLOYD of a similar alteration in the abscission of floral organs. Investigation of the more fundamental factors underlying the ultimate chemical changes discussed in this paper must be made before general conclusions of the causes leading up to abscission can be drawn. The significance of the presence of an abundance of nitrates in the abscission layer at the time of abscission is uncertain. Their ability to affect the water holding capacity of colloids and similar effects of other ions which are changing in concentration in this region may influence the permeability of the cell membranes of these cells, a question that has not yet been touched upon experimentally.

Conclusion

Abscission of leaves in *Coleus Blumei* is a result of the conversion of cellulose into pectose, which is further transformed to pectin and pectic acid, leading to the formation of an excess amount of pectic acid over that of the available calcium sufficient to maintain the solidity of the middle lamella of the cell walls of the abscission layer. These processes are possibly initiated and probably accelerated by the presence of oxidases and ferric ions, both of which accumulate in the abscission layer.

Microchemical methods employed

In the microchemical study color reactions were used for orientation. These were followed by specific chemical reactions and solubility tests. A brief outline of the tests made for each substance follows. Details of these reactions may be found in recent microchemical texts.

CELLULOSE.—(1) Chlorzinc iodide: blue color; (2) hydrocellulose reaction: blue color with iodine after treatment with 75 per cent sulphuric acid; (3) solubility: insoluble in dilute acids and alkalies, soluble in copper-oxide-ammonia; (4) crystallization: dissolve in copper-oxide-ammonia, wash with ammonia and water; colorless sphaero crystals or spiculate crystal clusters appear within the cells; (5) crystal reactions: insoluble in dilute acids and alkalies, soluble in copper-oxide-ammonia and sulphuric acid; blue color with chlorzinc iodide; (6) membranes of cellulose exhibit double refraction in polarized light.

PECTIC COMPOUNDS IN GENERAL.—(1) Ruthenium red: red color; (2) methylene blue: violet color; (3) membranes of pectic compounds do not exhibit double refraction in polarized light.

PECTOSE.—(1) Insoluble in copper-oxide-ammonia, dilute alkalies, ammonia, and alkali carbonates; (2) converted into pectic acid and pectin when gently heated with 2 per cent hydrochloric acid for 30 minutes. These latter substances are readily dissolved by 2 per cent potassium hydroxide or 5 per cent sodium carbonate, leaving the cellulose membrane intact.

PECTIC ACID.—(1) Soluble in dilute alkalies, ammonia, and alkali carbonates; (2) insoluble in water.

PECTIN.—Soluble in water.

CALCIUM PECTATE.—(1) Hydrolyzed by 2 per cent hydrochloric acid: calcium chloride is formed and pectic acid set free; (2) 3 per cent oxalic acid or ammonium oxalate: calcium oxalate crystals are formed, pectic acid set free; (3) 5 per cent sulphuric acid: calcium sulphate crystals formed, pectic acid set free.

CALCIUM.—(1) Two per cent oxalic acid: calcium oxalate crystals; (2) 5 per cent sulphuric acid: calcium sulphate crystals.

LIGNIN.—Phloroglucin-HCl reaction: red violet color.

SUBERIN.—(1) Sudan III or Scharlach R: red color; (2) insoluble in copper-oxide-ammonia; (3) phellonic acid reaction.

FRUCTOSE.—(1) Fluckiger's reaction: yellowish-red precipitate of cuprous oxide at once without heating; (2) phenylhydrazine reaction: yellow osazone crystals formed in 6–8 hours; (3) methylphenylhydrazine reaction: insoluble osazone; crystals formed in 15 minutes if preparation is heated, after 24 hours at room temperature.

GLUCOSE.—(1) Fluckiger's reaction: yellowish-red precipitate of cuprous oxide after heating 1–2 minutes; (2) phenylhydrazine reaction: yellow osazone crystals formed after about 24 hours.

SUCROSE.—Remove fructose and glucose. Invert with hydrochloric acid. Test for glucose and fructose as preceding.

NITRATE.—(1) Diphenylamine sulphuric acid reaction: blue color slowly changing to brown-yellow; (2) brucin-sulphuric acid reaction: red color.

OXIDASES.—Benzedine reaction: blue or purple precipitate if tissue is acid, soon changing to brown; brown precipitate at once if tissue is neutral or alkaline.

IRON.—(1) Berlin blue reaction: sections in 2 per cent solution of potassium ferrocyanide 15 minutes, add a drop of 2 per cent hydrochloric acid. A dark blue precipitate indicates the presence of ferric ions. Similarly a red color with potassium ferricyanide indicates the presence of ferrous ions; (2) sodium thiosulphate: red color.

MANGANESE.—Sections in 0.1 per cent hydrochloric acid, add 0.5 per cent sodium ammonium phosphate and ammonia vapor: ammonium manganese phosphate crystals, brown color in a 2 per cent solution of potassium permanganate.

MALIC ACID.—(1) Silver nitrate: sphaero crystals of silver nitrate, soluble in ammonia; (2) lead oxide: lead malate crystals; (3) sublimation: concentrated sulphuric acid, heat to 130°C.; slight charring.

OXALIC ACID.—(1) Uranium acetate: large yellow crystals of uranium oxalate; (2) strontium nitrate: strontium oxalate crystals; (3) ferrous phosphate: yellow precipitate of ferrous oxalate.

AMINO ACIDS.—Crystallization: treat sections with absolute alcohol, crystals of amino acids; (1) compare with known crystal form; (2) specific reactions.

TYROSINE.—Millon reaction: red color.

ARGININE, HISTIDINE.—Picrolonic acid: yellow crystalline precipitate.

LEUCINE.—Sublimation at 170°C.

ASPARAGINE, GLUTAMINE.—Quinone: red color.

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